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"De Novo" Duplication Xq23→Xq26 of Paternal Origin in a Girl With a Mildly Affected Phenotype

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We report a de novo dup(X)(q23→q26) in a 3-year-old girl with growth retardation, developmental delay, and minor anomalies. X-inactivation in lymphocytes by BRDU labeling showed the abnormal X was late replicating. The androgen receptor assay (HAR) demonstrated a skewed methylation (88.8%) of the paternal allele and a 11.2% methylation of the maternal allele. These data, which suggest the duplication was paternally inherited, are the first parental-origin identification of a duplication Xq. The mild phenotype of the patient may be related to the size and region of the duplication, the low percentage of a dup(X) active detected by the HAR assay, or a combination of these mechanisms. Am. J. Med. Genet. 70:404–408, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: duplication Xq; X-inactivation; androgen receptor locus assay (HAR); paternal origin

INTRODUCTION

Duplications of the long arm of chromosome X can be inherited (familial) or de novo. Inherited duplications have been detected more frequently, segregate for several generations transmitted by female carriers who usually are normal or nearly normal (with only short stature), and have been ascertained through malformed male offspring [Aughton et al., 1993]. De novo duplications are rather rare and so far always have been associated with abnormalities such as Ullrich-Turner syndrome, gonadal dysgenesis [Varella-Garcia et al., 1981; Van Dyke et al., 1983; Knuutila et al., 1984], or more often congenital anomalies and mental retardation [Aughton et al., 1993]. It is not known if these differences have implications for follow-up and genetic counseling or they reflect an ascertainment bias since symptomatic females are more likely to be diagnosed.

In this paper we present a paternally derived de novo duplication Xq23→q26 in a girl with developmental delay, growth retardation and other minor anomalies. The paternal origin was demonstrated through X-inactivation studies in blood cells with the androgen receptor assay and the late replication technique by BRDU.

CLINICAL REPORT

Clinical Manifestations

KG was evaluated at birth because she was small for gestational age. The pregnancy was uncomplicated and KG was born at term by caesarian section due to placenta previa. Birth weight was 43 cm, length was 1,984 kg and OFC was 30 cm. KG was discharged with her mother after an uncomplicated hospital stay.

The family history was unremarkable, and the proposita has two normal living sibs.

Examination at 1 month showed dolichocephaly, large anterior and posterior fontanelles, partial 2–3 syndactyly of the toes, clenched fists, and length, weight, and OFC below the 5th centile.

At 6 months, her growth parameters remained below the 5th centile; the posterior fontanelle was patent and the anterior fontanelle was large. A single bilateral bridged palmar crease was noted.

At 18 months the fontanelles were closed. She had bilaterally adducted thumbs that did not compromise motility or motor function.

The most recent evaluation at 3 years showed growth parameters below the 5th centile with the head size being proportionately small for her size, downslanted palpebral fissures, and bilaterally adducted, hypoplastic thumbs (Figs. 1, 2, and 3).

KG received early childhood intervention services until age 3 years. She walked at 15 months. At 32 months she was not combining words yet. By 3 years she was using short sentences and scribbling. She is receiving speech therapy and is in a Head Start Program.

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Fig. 1. The proposita at age 3 years.

Cytogenetic Studies

Chromosome analysis in blood lymphocytes by GTG banding showed a direct duplication Xq23→Xq26 (Fig. 4) (550–600 bands level) [karyotype: 46,XX,dir dup(X)(q23→q26)]. It was considered de novo because parental chromosomes were normal.

Fluorescent in situ hybridization (FISH) using the CISS technique [Pinkel et al., 1988] with a chromosome X library (Cambio Laboratories, Cambridge, UK) demonstrated that the extra bands were from chromosome X (Fig. 5).

Fig. 2. Abducted and hypoplastic thumb.

Fig. 3. Increased gap between toes 1 and 2 with 2–3 partial syndactyly.

X-inactivation was studied in 50 metaphases from blood lymphocytes using the BRDU labeling technique in late S-phase developed by Dutrillaux et al. [1976]. Cytogenetic confirmation and X-inactivation studies could not be done in fibroblasts because the family did not give consent for a skin biopsy.

Androgen Receptor X-Inactivation Assay

The human androgen receptor assay (HAR) was used because the methylation of HpaII sites in the first exon of the androgen receptor locus (Xq11.2-Xq12) correlates with X-inactivation. This is a highly informative PCR assay (90% heterozygosity) that distinguishes each parental X chromosome based on the methylation of a CpG region adjacent to a polymorphic CAG repeat [Allen et al., 1992]. The HAR data were correlated with the cytogenetic analysis of X-inactivation to identify the parental origin according to an established protocol [Martin et al., 1995].

DNA from the patient and both parents was isolated from blood leukocytes and the HAR test was performed as previously described [Pegoraro et al., 1994].

The relative sizes of the PCR product, amplified from the androgen receptor gene alleles, as indicated by the major peaks, were assessed visually.

RESULTS

Molecular and Cytogenetic Analysis of X-Inactivation

The BRDU labeling in lymphocytes showed preferential inactivation of the dup (X) in all metaphases (50 cells) (Fig. 6). Similar results were observed in lymphoblasts.

The androgen receptor assay (HAR) identified each parental X chromosome and the maternal and paternal alleles inherited by the proposita (Fig. 7). The father had a single peak (o, Fig. 7C). Both the patient and her mother had two peaks corresponding to one allele each (* and o, Fig. 7B; * and +, Fig. 7D). The patient showed

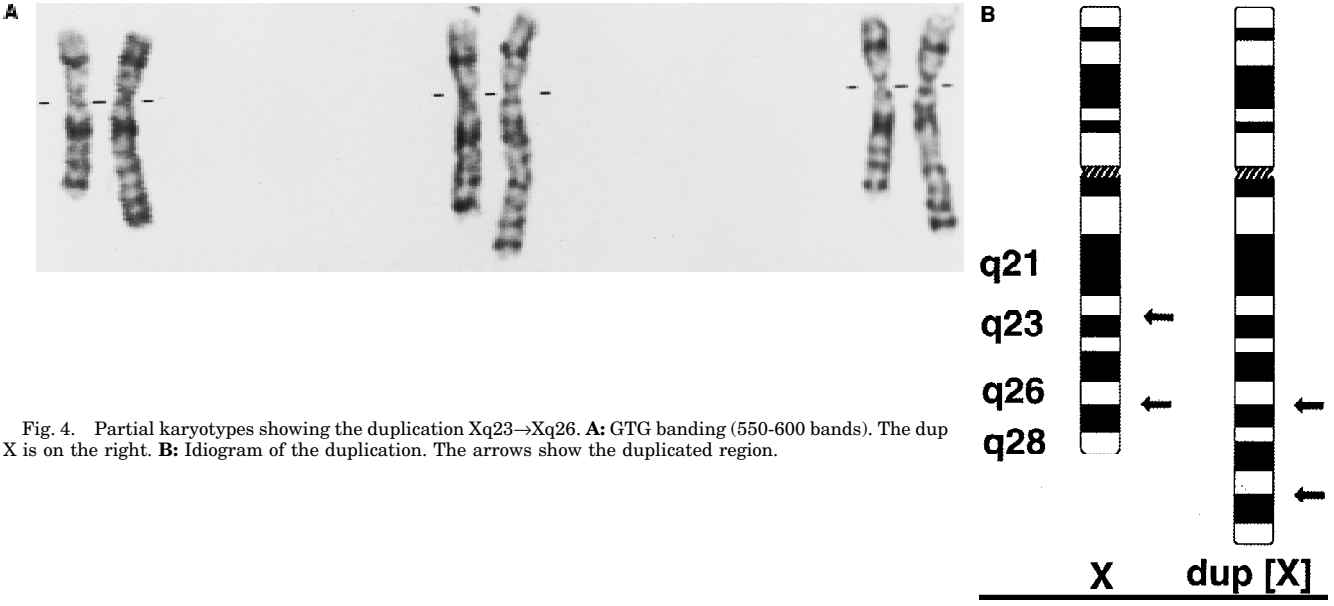

Fig. 4. Partial karyotypes showing the duplication Xq23→Xq26. **A:** GTG banding (550-600 bands). The dup X is on the right. **B:** Idiogram of the duplication. The arrows show the duplicated region.

a skewed methylation (88.8%) of the paternal allele (o) in blood leukocytes DNA digested with HpaII and CfoI. The maternal allele (*) was 11.2% methylated (Fig. 7A).

Since the dup(X) is late replicating and the paternal HAR allele (o) is preferentially methylated (88.8% inactive), these data suggest a paternal origin of the duplication.

DISCUSSION

We have described a 3-year-old girl with a de novo dup(X)(q23→q26) detected by GTG banding and confirmed by FISH. Her growth retardation, developmental delay and minor anomalies were considered a mild phenotype compared to most previous cases (Table I). Molecular-cytogenetic studies of X-inactivation, which demonstrated the duplication was inherited from the father, are the first identification of parental origin.

With the exception of three cases with Ullrich-Turner syndrome or gonadal dysgenesis [Varella Garcia et al., 1981; Van Dyke et al., 1983; Knuutila et al., 1984] duplications of Xq lack a characteristic phenotype (Table I). Limited comparisons could be made with triple X females, although this syndrome is clinically variable due to methods of ascertainment. Minor craniofacial anomalies have been observed in triple X females [Ballesta and Zapata, 1989] and in de novo duplications Xq referred to genetic evaluation (cases l, 2, 4, and the present case; Table I). Prospective follow-ups in triple X newborn infants ascertained by neonatal screening have shown motor and developmental delay, learning disabilities, and behavior or emotional problems [Robinson et al., 1992]. Difficulties with language, neuromotor, and learning skills with poor psychosocial

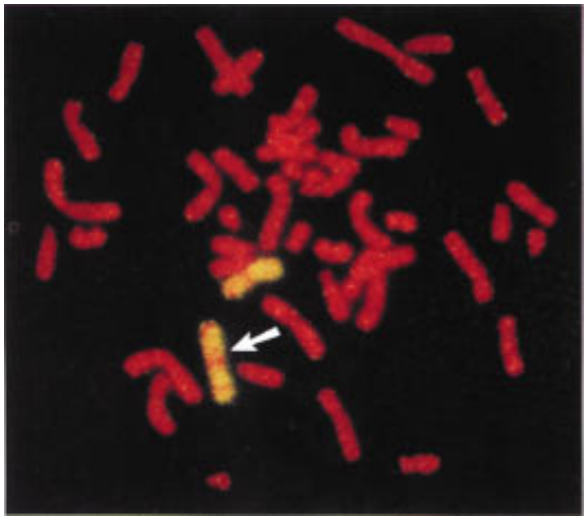

Fig. 5. FISH painting with chromosome X library. The arrow shows the duplicated chromosome X.

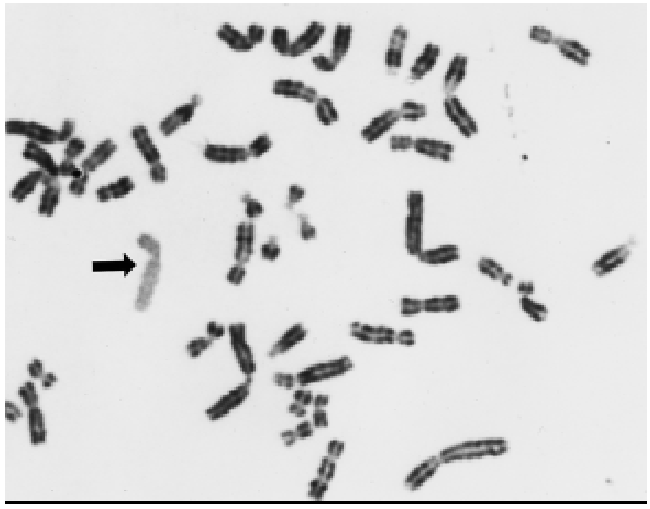

Fig. 6. Metaphase that shows the late-replication duplicated X by the BRDU labeling technique in late S-phase.

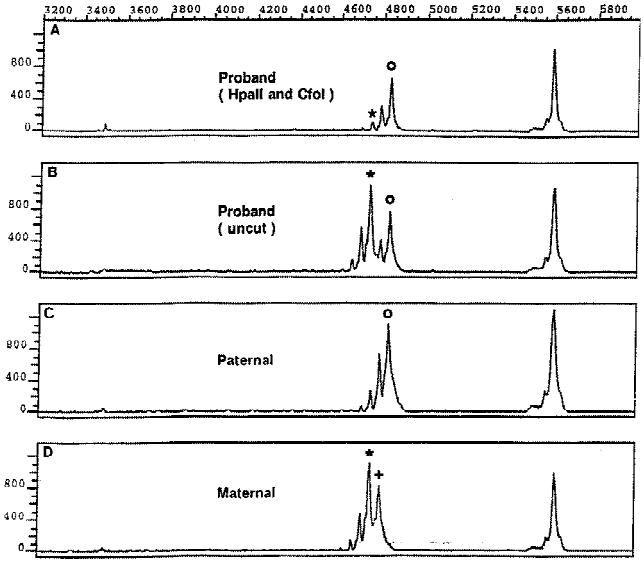

Fig. 7. X-inactivation profiles in the proposita and both parents by the human androgen receptor assay (HAR) in blood leukocytes. **A**: The paternal allele (o) is preferentially methylated (88.8%). The small peak (*) is the maternal allele (11.2% methylation). **B**: Uncut DNA showing the maternal (*) and the paternal (o) alleles. **C**: The paternal profile shows the allele (o). **D**: The maternal DNA shows two distinctive alleles, (*) and (+).

adaptation were seen in some duplications Xq (cases 1, 4, 5, and the present case; Table I).

X-inactivation could have significant clinical impact in females with de novo duplications Xq.

The dup (X) is consistently late replicating in lymphocytes and fibroblasts, so this cytologically inactive status does not correlate with the abnormal phenotype [Aughton et al., 1993]. It may be argued the cytogenetic techniques were not sensitive to detect a low percentage of cells with a normal X inactive in these cases or that late replication does not necessarily imply genetic inactivity because some genes escape inactivation [Willard, 1995]. It is also possible that a higher Xq gene dosage disrupted early embryogenesis or that lymphocytes and fibroblasts are not representative of other tissues, which could have a different X-inactivation. In our case it is quite relevant that the HAR test detected a 11.2% methylation of the maternal allele carried by the normal X [so the dup(X) is active in these cells], but late replication failed to show it is inactive. This would suggest that late replication with BRDU was not sensitive for X-inactivation analysis, while the HAR test is more accurate and most likely the active duplicated X is associated with the abnormal phenotype. These findings raise questions about the role of X-inactivation that could be answered by a systematic molecular analysis in different tissues from the same patient. It would also be important to study familial duplications involving regions similar to de novo cases to rule out bias in ascertainment. The human androgen receptor assay is suitable for this testing because different tissues can be easily studied by PCR, it is very informative and sensitive to identify each chromosome X based on the methylation of paternal and maternal alleles, it has the additional advantage of determining parental origin combined with late replication by BRDU [Martin et al., 1995], and it yields data that correlate with X-inactivation [Allen et al., 1992].

Parental origin could also be another factor that modulates the phenotype in de novo duplications Xq but there are not data yet. Our case is the first report, so final conclusions cannot be drawn but it would be interesting to compare maternal and paternal cases. These studies could also determine if the maternal and paternal X are equally susceptible to these rearrangements or there are differences depending on parental origin similar to de novo balanced and unbalanced X-autosome translocations, which are mostly paternal [Garcia-Heras et al., 1997].

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TABLE I. Summary of Clinical Findings in Females With De Novo Duplications Xq

Reference	Duplicated region	Phenotype
1 Aughton et al. [1993][a]	q13→qter	Developmental delay; severe seizures and multiple minor anomalies
2 Varella-Garcia et al. [1981][b]	q21→q27	MR; malformations and signs of pure gonadal dysgenesis
3 Van Dyke et al. [1983][c]	q13.3→q27.2	Signs of Ullrich-Turner syndrome
4 Knuutila et al. [1984][d]	q21.1→q24	MR (IQ 30); developmental and speech delay, craniofacial anomalies and gonadal dysgenesis
5 Crandall et al. [1993][e]	q22→q28	Short stature, growth retardation and behavior problems
6 Present case[f]	q23→q26	Developmental and speech delay; growth retardation and few minor anomalies

[a]IUGR, low birth-weight, growth retardation, deeply-set eyes, epicanthal folds, telecanthus, prominent/broad nasal bridge, bulbous nasal tip, long philtrum, small mouth, short neck, broad chest, diastasis of recti, puffy hands and feet, short 1st metacarpals, hypotonia, seizures, and diffuse "salt and pepper" retinopathy.
[b]Hypertelorism, telecanthus, high palate, prognathism, broad nasal bridge, widely spaced nipples, poorly developed breasts, absent axillary hair, sparse pubic hair, genu valgum, equinovarus, cubitus valgus, 3–4 camptodactyly, dorsal kyphoscoliosis, and uterine hypoplasia/streak gonads.
[c]Immature and small face, short stature, gonadal dysgenesis, secondary amenorrhea, low posterior hairline, telangiectasis in trunk and lips, ovarian streaks, and small uterus.
[d]Acrocephaly, flat occiput, coarse face, webbed neck, broad chest, widely-spaced nipples, poor breast development, and hypotonia.
[e]Growth retardation with short stature, microcephaly, optic nerve hypoplasia, asymmetry in the body and extremities, cognitive, behavioral and psychosocial problems, learning disabilities, and fine motor incoordination.
[f]Dolichocephaly, downslanted palpebral fissures, partial 2–3 toe syndactyly, single bilateral palmar crease, and bilateral adducted and hypoplastic thumbs.

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